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EXPLORING EMS AND SODIUM AZIDE-INDUCED MUTAGENESIS IN VICIA FABA L.

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ABSTRACT

Induced mutagenesis is a valuable tool in plant breeding, offering a method to enhance desirable traits and introduce new characteristics in crops. This research focuses on the effects of Ethyl Methanesulfonate (EMS) and Sodium Azide (NaN3)-induced mutagenesis in *Vicia Faba*, a legume recognized for its nutritional value and adaptability. *Vicia Faba* has the potential to contribute to solutions for global food security challenges. EMS and NaN3 are mutagenic agents that induce genetic variation, which can lead to crops with improved traits. However, a deep understanding of the mutagenic mechanisms and a comprehensive characterization of induced mutations are essential for maximizing the benefits of these agents. This study aims to explore the effects of EMS and NaN3 on *Vicia Faba* at the morphological, biological, and cytological levels. By understanding the impacts of these mutagens, the research will offer insights into the processes behind induced genetic variation. Such insights are crucial for optimizing mutagenesis in plant breeding programs. The goal of the project is to contribute to the refinement of breeding strategies, focusing on creating more resilient and high-yielding crop varieties.

KEYWORDS: EMS, Sodium Azide, *Vicia Faba*, Chromosomal Aberrations, Induced Mutagenesis

INTRODUCTION

This research project aims to investigate the effects of EMS and SA-induced mutagenesis on *Vicia Faba* at both the cytological and agronomic levels. By integrating phenomic and mitotic approaches, the study seeks to uncover insights into the genetic architecture of key agronomic traits in *Vicia Faba* and accelerate breeding efforts for varieties with improved resilience and productivity.

Induced mutagenesis involves the deliberate use of mutagenic agents, such as chemicals or radiation, to generate genetic variation, enhancing desirable traits or introducing new characteristics in plants (Bhat et al., 2017). *Vicia Faba* L., commonly known as broad bean or faba bean, holds significant nutritional and economic importance worldwide, making it a major research focus in agricultural sciences (Zong et al., 2020). This leguminous crop is valued for its high protein content, essential amino acid profile, and role in human dietary diversity and food security (Hall et al., 2017). Additionally, *Vicia Faba* contributes to soil fertility through nitrogen fixation via symbiotic relationships with rhizobia, promoting sustainable cropping systems (Herridge et al., 2008).

Among mutagens, Ethyl Methanesulfonate (EMS) and Sodium Azide (SA) have gained attention for inducing mutations in plant genomes. EMS induces point mutations by alkylating DNA bases, leading to nucleotide substitutions (Shah et al., 2016), while NaN3 causes DNA damage, including base pair substitutions and frame-shift mutations (Khan et al., 2009; Gautam et al., 2016). Several studies have reported dose-dependent reductions in biological parameters after mutagenic treatments (Bhat et al., 2006; Jafri et al., 2011; Khan et al., 2009), and Kumar et al. (2019) noted that induced mutagenesis can rapidly generate genetic variability, overcoming natural variability constraints. However, mutagenesis can also result in physiological imbalances or chromosomal anomalies, leading to reduced plant survival (Rao & Rao, 1983). Khan et al. (2015) observed variations in plant traits and stress resistance in mutagenized *Vicia Faba* populations.

MATERIALS AND METHODS:

The standard variety of *Vicia Faba* L. used in this study consisted of certified, dry, healthy seeds obtained from the Government seed store in Aligarh, Uttar Pradesh, India. The mutagenic agents employed were Ethyl Methanesulfonate (EMS) and Sodium Azide (SA). EMS, known for inducing random mutations via nucleotide substitution, and SA, an odorless white crystalline solid, both help induce genetic variations in *Vicia Faba*.

Stock solutions of EMS and SA were prepared at concentrations of 1% (v/v) for EMS and 1% (w/v) for SA in phosphate buffers with a pH of 7.0. This preparation method, based on the work of Hasan et al. (2022) and Naaz et al. (2023), aimed to optimize mutagenic efficiency while minimizing negative effects on seed viability and germination. EMS was prepared as a 1% (v/v) solution, and NaN3 was prepared at concentrations of 0.01% to 0.05%.

Six seeds per treatment were placed in Petri dishes and incubated at 27±1°C. Three replicates of six seeds each were also planted in 9-inch pots filled with a mixture of farmyard manure, soil, and sand (1:1:1 ratio). Planting occurred in mid-October 2022, corresponding to the rabi season. Pollen viability was assessed using 2% acetocarmine staining, while meiotic

studies were conducted on young flower buds using Carnoy's fixative (Jafri, 2009; Khan, 2015).

STATISTICAL ANALYSIS

The quantitative traits of the M1 generation were carefully analyzed using standard statistical methods to understand the extent and type of genetic changes induced. Mean standard error, standard deviation, and coefficient of variation were calculated following established procedures.

RESULTS & DISCUSSION

Biological and Morphological Parameters Studied in The M1 Generation:

The effects of mutagenic treatments, Ethyl Methanesulfonate (EMS) and Sodium Azide (SA), on various biological and morphological parameters were assessed in the M1 generation of *Vicia Faba*.

Biological Parameters:

1. **Seed Germination (%)**
   The highest seed germination was observed in the control group, with a baseline germination percentage of 92.85%. After exposure to increasing concentrations of EMS and SA, seed germination declined. For EMS, germination decreased from 92.85% (control) to 66.66%, and for SA, from 90.00% to 72.22%. These results highlight the dose-dependent inhibitory effects of EMS and SA on germination, consistent with Laskar & Khan (2013) and Akhtar et al. (2012).

2. **Seed Inhibition (%)**
   As germination decreased, seed inhibition increased. In EMS-treated seeds, inhibition ranged from 1.33% to 29.16%, while SA-treated seeds showed inhibition from 4.36% to 23.26%. These findings suggest that both mutagens hinder seed germination due to changes in DNA structure and function (Akhtar et al., 2012).

3. **Plant Survival (%)**
   Plant survival decreased with increasing mutagenic concentrations. The control group had a survival rate of 94.11%, while EMS-treated plants exhibited survival rates from 86.66% to 60.00%, and SA-treated plants showed rates from 84.60% to 55.55%. These results indicate that both mutagens negatively affected plant survival, with more severe effects at higher concentrations.

4. **Pollen Fertility (%)**
   Pollen fertility was high in the control group, but exposure to EMS and SA led to a dose-dependent decrease in fertility. For EMS-treated plants, pollen fertility decreased from 83.47% to 58.13%, while for SA-treated plants, it dropped from 74.56% to 51.25%. These findings suggest mutagen-induced reproductive abnormalities or disruptions in gametogenesis, as seen in studies by Akilan et al. (2019) and Azad (2013).

Morphological Parameters:

1. **Plant Height (cm)**
   The control group had an average plant height of 53.2 cm (Smith et al., 2019). EMS treatment produced a biphasic response. At lower concentrations (0.1% to 0.2%), plant height increased to 60.2 cm and 66.4 cm, suggesting a hormetic effect. However, at higher concentrations (0.3% to 0.5%), plant height declined, ranging from 58.6 cm to 42.6 cm. SA treatment led to a steady decrease in plant height from 56.2 cm to 42.6 cm, with a slight increase at 0.03% (52 cm).

2. **Branches per Plant**
   In the control group, plants had an average of 2.28 branches per plant. EMS treatment showed a biphasic effect, with a slight decrease in branches at lower concentrations (0.1% EMS) followed by an increase at 0.2% EMS. At higher concentrations (0.3% to 0.5%), branch numbers declined. SA treatment resulted in a similar pattern: an initial increase at 0.01% to 0.02%, followed by a decrease at higher concentrations.

3. **Pods per Plant**
   The average number of pods per plant in the control group was 3.56. EMS treatment caused a transient increase in pod numbers at lower concentrations (0.1% to 0.2%), followed by a decrease at higher concentrations (0.3% to 0.5%). SA treatment led to a steady increase in pods at lower concentrations (0.01% to 0.02%), with a decrease at higher concentrations. The coefficient of variation was higher in treated groups compared to the control.

4. **100 Seed Weight (g)**
   The control group had an average 100-seed weight of 42.63 g. EMS treatment showed a biphasic effect, with a slight increase in seed weight at lower concentrations (0.1% to 0.2%), followed by a decrease at higher concentrations (0.3% to 0.5%). SA treatment caused a progressive decrease in seed weight across concentrations, suggesting a dose-dependent effect on seed development. The coefficient of variation increased with higher concentrations compared to the control.

5. **100 Seed Yield (g)**
   The control group had a 100-seed yield of 56.0 g. EMS treatment initially enhanced yield at lower concentrations (0.1%-57.46 g), followed by a decline at higher concentrations (0.2%-53.91 g to 0.5%-48.20 g). Similarly, SA treatment caused a progressive decrease in seed yield (55.55 g to 47.80 g), reflecting a dose-dependent impact on reproductive efficiency.

Leaf variations included unifoliate, bifoliate, multifoliate, lobed, notched, and both regular and irregular shapes. Seed morphology also exhibited variations in size, color (yellow, brown, purple, black), and shape (flat, angular, or spherical), reflecting the diverse mutational outcomes induced by EMS and SA treatments.

Cytological parameters:

Cytological analysis revealed chromosomal aberrations induced by EMS and SA. In the control group, *Vicia Faba* exhibited the normal chromosome configuration of six perfect bivalents (2n=2x=12). However, plants from treated seeds showed meiotic disturbances, including multivalents, chromosomal stickiness, precocious separation, laggards, and unequal separation during anaphases I/II. Telophase I/II showed disturbed polarity, micronuclei formation, and multinucleate conditions. These chromosomal aberrations were dose-dependent, indicating the mutagenic potential of EMS and SA, in line with findings by Khan et al. (2015), Rubinaperveen et al. (2013), and Khursheed et al. (2015).

CONCLUSION

In this study, the effects of Ethyl Methanesulfonate (EMS) and Sodium Azide (SA) on various biological, morphological, and cytological parameters in *Vicia Faba* L. were thoroughly investigated. The results revealed significant alterations induced by these mutagens, reflecting their potency in inducing genetic variations and disturbances in plant development and reproductive processes. Aberration frequency showed a direct relationship with the dose mutagen used. EMS (0.1% and 0.2%) and SA (0.02%) with moderate effects were considered to be suitable for induced mutagenesis. Among them Ethyl methyl sulphonate is found to be more effective in induction of desirable characteristics than Sodium azide in *Vicia Faba* L. By Optimal usage of mutagens, breeders can accelerate the development of resilient and high-yielding crop varieties capable of meeting the challenges of a rapidly changing agricultural landscape.

Treatment	Plant height (cm)			No. of Branch per Plant			No. of pods per plant			Pod length (cm)
	X±SE	SD	C.V	X±SE	SD	C.V	X±SE	SD	C.V	X±SE
CONTROL	53.2±1.40	2.77	5.21	2.28±0.20	0.46	20.51	3.56±0.46	1.03	48.22	4.15±0.51
EMS (%)										
0.1	60.2±1.52	3.42	5.68	2.25±0.47	1.05	46.90	2.81±0.52	1.16	41.43	5.00±0.40
0.2	66.4±1.74	3.91	6.89	2.91±0.48	1.08	37.15	3.25±0.95	2.14	65.90	4.65±0.55
0.3	58.6±1.07	2.40	4.10	2.78±0.35	0.80	28.78	3.06±0.66	1.48	48.47	3.71±0.68
0.4	42.6±0.81	1.81	4.26	2.55±0.39	0.88	34.50	2.93±0.68	1.53	52.28	4.00±0.68
0.5	56.2±1.74	3.89	6.93	2.40±0.22	0.50	21.12	2.86±0.73	1.64	57.26	4.55±0.69
SA (%)										
0.01	56.2±1.59	3.56	6.34	2.00±0.28	0.03	31.62	2.46±0.58	1.30	52.78	3.26±0.32
0.02	41.0±1.59	3.57	8.72	2.33±0.34	0.77	33.36	3.60±0.50	1.14	31.67	4.41±0.58
0.03	52.0±1.67	3.74	9.12	2.14±016	0.36	16.94	3.06±0.93	2.08	68.02	3.63±0.65
0.04	42.6±1.56	3.50	8.59	2.50±0.23	0.52	21.08	2.44±0.72	1.63	66.94	4.20±0.53
0.05	42.6±1.78	4.0	9.75	2.00±0.43	0.96	48.30	2.41±0.86	1.92	79.80	3.56±0.59

Table 1. Effect of EMS and SA on plant height, no. of branches per plant, no. of pods per plant and pod length in V*icia faba* L. (M_1 Generation).

Treatment	Weight of 100 seeds/plant (gm)	Total yield/plant (gm)	Pollen sterility
	X±SE	X	(%)
CONTROL	42.63±0.12	56.06±0.64	-
EMS (%)			
0.1	43.06±0.57	57.46±0.47	7.79
0.2	42.28±0.18	53.91±017	15.69
0.3	41.56±0.43	52.60±0.35	20.66
0.4	40.78±0.69	49.80±0.72	29.97
0.5	39.32±0.94	48.20±0.22	35.78
SA (%)			
0.01	41.66±0.30	55.55±0.28	17.64
0.02	40.04±0.78	52.33±0.65	24.59
0.03	40.64±0.15	49.14±0.61	32.40
0.04	39.43±0.61	47.57±0.23	37.66
0.05	39.40±0.43	47.80±0.43	43.38

Table 2. Effect of EMS and SA on weight of 100 seeds/plant and total yield per/plant and pollen sterility in *Vicia faba* L. (M_1 generation).

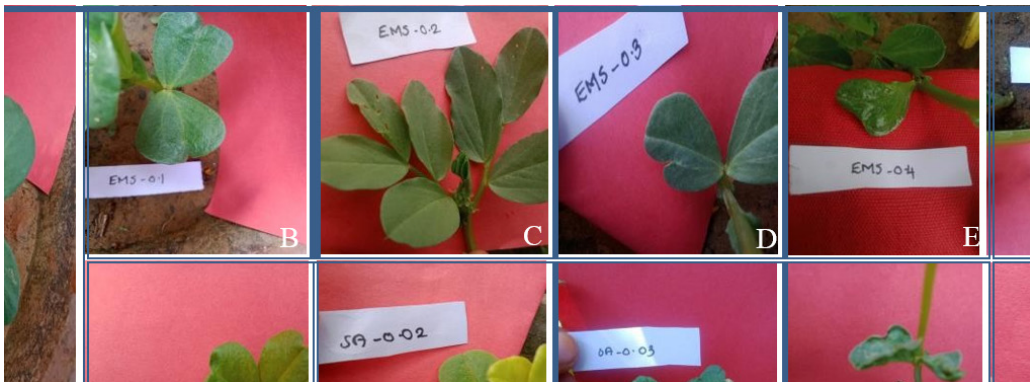

Fig.1: Variations of leaves: A Vicia Faba L. (Control). B&D Bifoliate notched leaves in 0.1% & 0.3% EMS. C Multifoliate leaves in 0.2 % EMS. E&F Unifoliate leaf in 0.4% & 0.5% EMS. G&H Bifoliate notched leaves in 0.01% & 0.02% SA. I Bifoliate irregular leaves with a giga and small leaf in 0.03% SA. J Bifoliate irregular curled leaves in 0.04% SA. K Unifoliate leaf in 0.05% SA.

Fig.2: Seed abnormalities: variation in color and shape A Medium sized purple seed. B Small dark brown seed. C Medium yellow seed. D Large flat seed. E Spherical black seed. F Angular green seed.

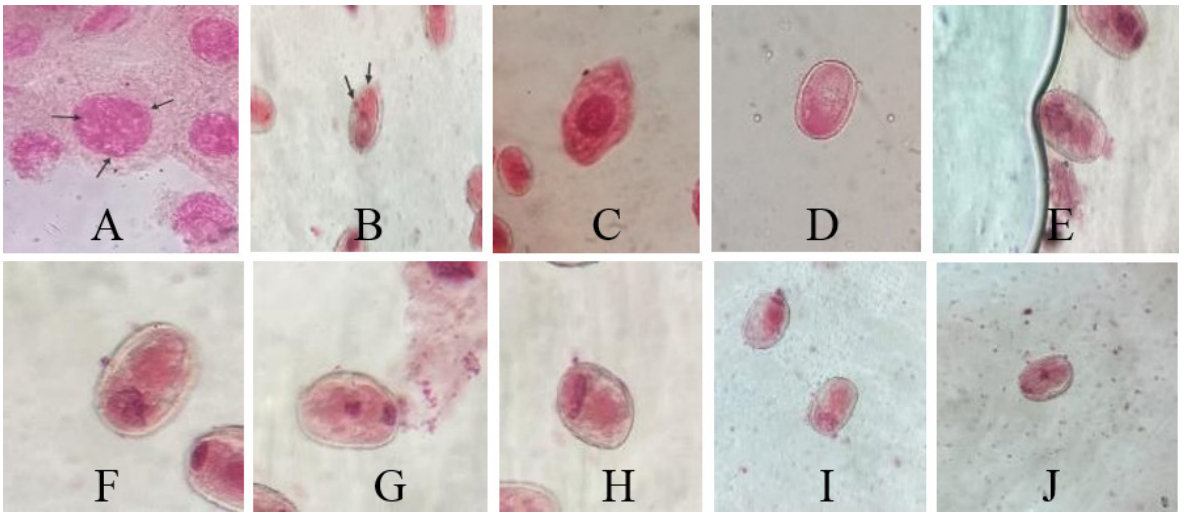

Fig.3: Cytological stages identified at higher concentration of mutagens: A PMC showing Multinucleated cells in (EMS 0.5%). B PMC showing fragments at anaphase I (SA 0.05%). C PMC showing binucleated condition (EMS 0.4%). D PMC showing metaphase I (EMS 0.2%). E PMC showing laggards at anaphase I (SA 0.05%). F PMC showing disturbed metaphase (SA 0.05%). G PMC showing sticky telophase at (SA 0.04%). H PMC showing stickiness in metaphase l (SA 0.04%). I PMC showing disturbed metaphase l (EMS 0.4%). J PMC showing laggards at anaphase 1 EMS (0.5%)

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